

Interspecific Interactions and Insecticide Applications Shape Pest Community
Structure on Eastern Hemlock

An Honors Thesis for the Environmental Studies Program.

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Introduction

Throughout my life, I have felt at home in the outdoors. There are few things that bring me a greater feeling of peace than stepping into dark, quiet woods and just simply walking. Conifers, in particular, stand out to me – their distinctly sharp and clean scent, along with the shade stretching beneath their needled branches, are always a welcome addition to any environment I find myself in. In the forests of New England and upstate New York, where I've done much of my hiking and exploring, there exists one tree in particular which I hold quite dear. The eastern hemlock (*Tsuga canadensis*) is a slow-growing, shade-tolerant, coniferous evergreen which can be found in cool, damp regions, often near water bodies (Abella 2014). Growing up, I adored this tree and wished it would one day become more widespread. The current state of the eastern hemlock, unfortunately, does not reflect this hope in any way.

The introduction of two herbivorous insects, the hemlock woolly adelgid (*Adelges tsugae*; HWA) and the elongate hemlock scale (*Fiorinia externa*; EHS) from Japan has resulted in a variety of adverse effects on eastern hemlock in the eastern United States, leading to its severe decline in much of the region (Orwig and Foster 1998; Danoff-Burg and Bird 2002; Orwig et al. 2002; Orwig et al. 2008; Miller-Pierce et al. 2010; Gómez et al. 2014). There is a large body of research on the biology of HWA and EHS, as researchers have sought to gain an understanding of the pests and how they may be managed. HWA is regarded as the more destructive of the two, as it drastically reduces hemlock growth (Orwig and Foster 1998; Preisser et al. 2007; Miller-Pierce et al. 2010; Gómez et al.

2014). Both are passively dispersed, often transported long distances by the wind (McClure 1977; McClure 1989a; McClure 1990) and by birds, deer and humans (McClure 1990).

Attack by these two pests has contributed to significant loss of eastern hemlock in New England forests and shifts in ecosystem functioning. As hemlocks decline, regeneration is negligible, and faster-growing species such as black birch often take their place in the forest (Orwig and Foster 1998; Stadler et al. 2005). Greater amounts of light are allowed to reach the understory as dead needles drop from branches and the foliar area of hemlocks is reduced (Orwig and Foster 1998; Jenkins et al. 1999; Stadler et al. 2005; Orwig et al. 2008). This often results in drastic changes to the composition of plant communities in the understory, as smaller, herbaceous plant species thrive in the increased sunlight available to them (Orwig and Foster 1998; Stadler et al. 2005; Ribbons 2014). In addition, changes to the rates of decay and other dynamics of litter on the forest floor are common (Cobb 2010), including reduced long-term amounts of organic matter, reduced soil moisture (Orwig et al. 2008) and the short-term addition of greater amounts of fallen, woody debris as trees are killed (Orwig and Foster 1998). Availability and turnover of nitrogen in ecosystems may be increased as a result of these altered dynamics (Jenkins et al. 1999; Stadler et al. 2005; Orwig et al. 2008; Cobb 2010). Soil is not the only thing impacted, as streams are often found in hemlock stands, and such streams may support a great diversity of species that could not be supported by streams in other types of forest ecosystems (Snyder et al. 2002). Thus, loss of hemlock stands due to HWA will likely result

in reduced diversity in contained and nearby water bodies. Currently, the extent of the damage has been limited by extreme winter temperatures, which restrict the northward range expansion of these insects (McClure 1989a; Paradis et al. 2007). However, EHS has been shown to adapt to cold temperatures (Preisser and Elkinton 2008), and perhaps more concerning, climate change is likely to remove this barrier to expansion. As winter temperatures become milder (Paradis et al. 2007), allowing HWA and EHS to spread throughout the entirety of the eastern hemlock's North American range (Dukes et al. 2009).

In addition to causing widespread changes to ecosystem functioning, the disappearance of hemlocks in the northeastern United States has certainly affected humans as well. It has been estimated that the decline of hemlocks in New England is likely to lead to losses in property values of at least \$24.6 million (Li et al. 2014). This number is only a piece of the total social and economic losses related to hemlock decline, as it does not take into consideration the unquantifiable aesthetic and recreational value of the species to outdoor enthusiasts such as myself. In a review of the ways in which the decline of hemlocks may impact national parks, Abella (2014) pointed out visitor safety hazards and reduced overall park experience as two consequences. The Arnold Arboretum in Boston, MA has lost over 500 of their 1,900 approximated hemlocks (Arnold Arboretum 2016); resulting in noticeably thinner forests surrounding walking paths. As a frequent visitor to the Arboretum, I have felt firsthand the effects of such losses. There is simply no equivalent replacement for the eastern hemlock.

Because of the limited mobility of these pests, interspecific interactions in the system take on greater importance as HWA and EHS are confined to the tree on which they originally settle. When co-infesting a tree, the two herbivores compete for resources (Denno et al. 1995). EHS has been shown to be an effective competitor, often negatively affecting HWA densities (Preisser and Elkinton 2008; Miller-Pierce et al. 2012; Gómez, Gonda-King, Orians and Preisser 2014; Gómez et al. 2014). Furthermore, HWA avoids EHS when selecting within-host feeding sites (Gómez, Gonda-King, Orians and Preisser 2014). Conversely, it has been shown that EHS is not negatively affected by prior HWA settlement (Miller-Pierce and Preisser 2012). In systems where multiple herbivores share a common host, plants may exhibit different induced defenses depending on which pest attacks first, thus changing the dynamic of the interaction (Poelman et al. 2008; Erb et al. 2011). Indeed, there is evidence that feeding by HWA may alter the host plant's distribution and concentration of nutrients in such a way that is beneficial to EHS and increases its density (Danoff-Burg and Bird 2002; Preisser et al. 2007). Although interspecific interactions certainly affect each herbivore's density, it has also been shown that the effects of simultaneous feeding by these two insects can be different from what might be predicted based on the individual effects of each – in several studies, co-feeding by HWA and EHS was not as detrimental to hemlock foliage quality as feeding by HWA alone (Preisser and Elkinton 2008; Gómez et al. 2012). So, while HWA is probably the most direct threat to hemlock health, it is important to look at competition and other interspecific relationship dynamics that may be influencing adelgid densities on

hemlock. If the range of these pests expands northward as forecasted, newly- or soon-to-be- infested areas could have an opportunity not available to many regions previously infested by HWA: the opportunity to be proactive and to plan for the pest. Such a plan would certainly require informed decisions and a complete body of knowledge on all the different factors influencing its success, including the interactions between all pests in the system. To date, much of the research in this system has focused on the particular interactions between HWA and EHS. Meanwhile, another species has largely flown under the radar. Spruce spider mite (*Olygonychus ununguis*; SSM) is a non-specialist herbivore, native to North America, found on conifers including hemlock (Lizotte et al. 2012). Often studied due to their omnipresence as horticultural or agricultural pests (James and Price 2002; Szczepaniec et al. 2011; Lizotte et al. 2012; Smith et al. 2013; Alba et al. 2015; Gupta 2015), mites have only been studied in three instances in the context of eastern hemlock (Sclar et al. 1998; Raupp et al. 2004; Szczepaniec 2009), a relatively low number when their pervasiveness is taken into account. A common theme in research on mites is that applications of certain insecticides, particularly the neonicotinoid imidacloprid, have been shown to lead to outbreaks of SSM and other mites (Sclar et al. 1998; James and Price 2002; Raupp et al. 2004; Creary 2009; Smith et al. 2013; Gupta 2015). Incidentally, due to a lack of natural predators for HWA in the United States, imidacloprid is one of the preferred methods of controlling HWA populations (Webb et al. 2003; Cowles et al. 2006; Abella 2014; Benton et al. 2015). However, only one (Raupp et al. 2004)

of the three studies of SSM on hemlock investigated this connection between insecticide use and mite outbreaks.

When I first became involved with research in this system, the name “spruce spider mite” was not even familiar to me. I could have perhaps hazarded a guess that it was a miniscule creature, likely bothersome to humans in some way, that probably lives on spruce trees. What I never could have guessed is that I would be standing in the pouring rain in the middle of a Rhode Island forest, curiously inspecting an uncharacteristically gold-colored hemlock sapling, and hear the words “Spider mites!” exclaimed by a cold, wet, and coffee-bereft Dr. Robert Schaeffer. At the time I was doing field work at a site in Kingston, RI, and SSM was not an anticipated part of it. The site was an experimental, mixed conifer-deciduous forest located at Kraus Farm (see Gonda-King et al. 2014 for details on setup) and much of the research there was focused on the effects of feeding by HWA and EHS. Our objective on this particular day was to record density counts of the insects on all of the hemlock saplings at the site in order to measure the success of previous experimental inoculations. However, to the best of my knowledge, the trees were supposed to be green, not gold. The contrast between that one sapling and many of the others was so striking that I couldn’t help but wonder how I had not heard of spruce spider mites before. Who were these tiny mites, and if they could be found sharing a host with HWA and EHS in such great numbers, why were they not more commonly mentioned in the hemlock literature? Perhaps most surprising, why had almost no previous research looked at the connection between imidacloprid applications and mite outbreaks on

hemlock? It struck me as slightly disconcerting that land managers have selected a toxic insecticide as their weapon of choice to fight off infestations of HWA while perhaps not even understanding all of the possible non-target effects.

For this thesis, I set out to reach a better understanding of the interactions between HWA, EHS, and SSM, and how they play out both in naturally-occurring New England and in urban forests in the Greater Boston Area. The inclusion of both types of environments allowed me to study these interactions in two contexts: natural ecosystem functioning and direct impacts to humans, including the aesthetics of infested or dying hemlocks and disruption of commonly-used urban spaces. Through a series of studies I sought to fill in the knowledge gaps on current densities of the pests throughout New England, how changing densities of one pest may impact the populations of others, and unintended side effects might arise from the use of imidacloprid in pest control. It is my hope that this work may provide some answers while also encouraging future research and management efforts to retain a whole-systems approach when thinking about solutions to address these pests.

Chapter 1: Interactions between invasive and native herbivores in New England eastern hemlock forests

Introduction

Invasive herbivores introduced to novel regions, intentionally or unintentionally, often develop established ranges and become part of ecosystems in invaded regions (Pimental et al. 2005; Liebhold and Tobin 2008). Successful range expansion however is affected by a number of factors. Abiotic elements, such as topography and climate, may either facilitate or slow the spread of an invasive herbivore (Liebhold and Tobin 2008; Gómez et al. 2014), as does the presence and abundance of suitable host plants. While these factors influence all herbivores, invasives often have several advantages over native species within the invaded region. One distinct competitive advantage is the lack of effective predators in invaded areas (Preisser et al. 2007; Miller-Pierce and Preisser 2012; Gómez et al. 2014). Additionally, host plants do not have the benefit of a long history of co-evolution with the invasive herbivore, and the plant's defenses are often ill-equipped to deal with attack (Liebhold et al. 1995; Gandhi and Herms 2010; Gómez et al. 2014). However, this is not to say that invasive herbivores face no barriers to range expansion whatsoever, as competition may exist in the form of other herbivores – invasive or native – vying for the same food source (Gómez et al. 2014). Invasive competitors have become more common over time due to increased numbers of exotic species introduced to novel regions (Liebhold et al. 1995; Orwig et al. 2008; Gómez et al. 2014), and competition is more

common between invasive herbivores than between native species (Denno et al. 1995; Preisser et al. 2007). While competitive relationships occur most frequently, facilitative interactions have been documented as well (Preisser et al. 2007). In any case, the presence of other herbivores on a shared host plant must be taken into consideration when studying the spread of an invasive herbivore, as such interspecific interactions affect not only the herbivores but the host plant as well, which may be of greater ecological concern.

In eastern North America, one species of particular concern to ecologists is the eastern hemlock (*Tsuga canadensis*), a native conifer found throughout the region (Preisser et al. 2007; Gómez et al. 2014). Hemlocks are long-lived and shade-tolerant, and their deep canopies and especially acidic litter make them uniquely important contributors within many forest ecosystems (Jenkins et al. 1999; Stadler et al. 2005; Orwig et al. 2008; Cobb 2010). Many native species depend on hemlocks and they have become host to a pair of invasive insects in recent years as well (Snyder et al. 2002; Gómez et al. 2014). Hemlock woolly adelgid (*Adelges tsugae*; HWA) and elongate hemlock scale (*Fiorinia externa*; EHS), two herbivores introduced to the United States from Japan in the last century, have become widespread throughout eastern hemlock's North America range (Preisser et al. 2007; Orwig et al. 2008; Miller-Pierce and Preisser 2012; Gómez et al. 2014). Together, these two insects have caused extensive mortality in hemlock stands; while *F. externa* on its own is rarely lethal to its host, *A. tsugae* has been shown to kill mature trees in as little as 4 years (McClure 1991). Both herbivores are sessile feeders and are only able to spread passively, through

vectors such as wind, birds, deer and humans (McClure 1977; McClure 1989a; McClure 1990).

Because of their inability to select a new host once they settle, any biotic and abiotic factors influencing the success and distribution of sessile herbivores such as *A. tsugae* and *F. externa* are of great importance. The co-occurrence of these two insects is often detrimental to both, but over a longer period of time may result in higher densities of *F. externa* and lower densities of *A. tsugae* (Preisser et al. 2007), as well as less severe impacts to host plant health compared to the impacts of *A. tsugae* alone (Preisser and Elkinton 2008; Gómez et al. 2012). Additionally, abiotic factors such as topography (Orwig et al. 2002) and wind patterns (McClure 1977) may influence the likelihood of a given area becoming infested. Both herbivores are intolerant of extreme winter temperatures; as a result, climate too is a limiting factor to range expansion (McClure 1989a; Paradis et al. 2007).

While the relationship between *A. tsugae* and *F. externa* has been researched at length, no studies have specifically focused on the interactions between these two invasives and a native herbivore. Eastern hemlock also faces attack by the polyphagous, native, secondary pest spruce spider mite (*Oligonychus ununguis*; SSM). *Oligonychus ununguis* prefers cool weather; overwintering in eggs on the bark and needles of host plants (Lizotte et al. 2012). Feeding by *O. ununguis* causes chlorosis and permanent mottling of needles, and as such it is most often considered a horticultural pest due to the aesthetic damage it generates on ornamental trees (Lehman 1998; Lizotte et al. 2012). However,

infestations may cause needle drop and more severe damage or even death, especially in stressed or small trees (Lehman 1998). The dynamics of the relationships between *O. ununguis*, *A. tsugae*, and *F. externa* have not been explored, and the extent to which a mite infestation may facilitate or impede feeding, reproduction, and overall range expansion of the two invasives is unknown. Knowledge of these dynamics is important because of the unpredictability of combined effects compared to the effects of individual relationships; these effects may be non-additive such as the aforementioned mitigating effects of *F. externa* to *A. tsugae* damage on hemlock. The effect of one insect on its own might be mildly impactful, but its indirect effect (due to impacts on populations of another herbivore, for instance) might be substantially worse. In the case of *O. ununguis*, mite damage in itself can be of concern, but a possible scenario in which a mite infestation facilitates the spread of the much more harmful *A. tsugae* would have even greater ecological impacts. Alternatively, if *O. ununguis* in fact hinders the success of *A. tsugae*, either directly or perhaps by contributing to the spread of the competing *F. externa*, the resulting dynamic would certainly prove impactful as well.

To address these potential dynamics, in the present study, I conducted a landscape-level survey across a latitudinal transect through Connecticut, Massachusetts, Vermont and New Hampshire. I quantified the densities of the two invasives, *A. tsugae* and *F. externa*, as well as the native *O. ununguis*, and measured stand health at each surveyed site. The goal of this survey was to expand upon the results of previous landscape surveys that since 1997 have

monitored densities of *A. tsugae* and *F. externa*, as well as their effects on hemlock health, in a series of >120 stands across a latitudinal transect of southern New England (Orwig et al. 2002; Preisser et al. 2008, 2011; Orwig et al. 2012; Gómez et al. 2014). These surveys explored interspecific interactions between the two invasive herbivores and here I seek to gain additional insight into the expansion of their ranges over time by extending a subset of the previously surveyed stands for re-sampling. In addition, I strove to further understand the complex dynamics between exotic and native pests on a shared host by taking the first known measurements of *O. ununguis* densities on eastern hemlock in New England.

Methods

Stand location and site selection

In summer 2015 I conducted a survey of 45 eastern hemlock (*Tsuga canadensis*) stands along a 14,000 km² latitudinal transect of New England, USA, ranging from the Long Island Sound in Connecticut (CT) north to southwestern New Hampshire (NH) and southeastern Vermont (VT). Thirty of the 45 stands, those in Massachusetts (MA) and CT, constituted a randomly selected subset of stands previously sampled by Orwig et al. (2002) and Gómez et al. (2014). Detailed information on the original selection and characteristics of those stands can be found in Orwig et al. (2002). The 15 sites in NH and VT were selected using Google Maps. Criteria for site selection were designed to maximize the

likelihood that each site would contain a hemlock stand, and were as follows: (a) the site must be a piece of land shaded green (denoting park land, cemetery, or state/town forest), (b) the site must contain a flowing body of water, and (c) the site must lie within the selection region. The selection region consisted of all land bounded to the north by Charlestown, NH, and Bradford, NH; bounded to the east by NH Route 202; bounded to the south by the NH-MA border; and bounded to the east by Interstate 91. All sites meeting these criteria were pooled and 15 were randomly selected from the pool to be surveyed. 15 alternative sites were randomly selected as backups, since the existence of a hemlock stand at each site was supposed rather than guaranteed. For each site that did not contain a hemlock stand, an alternative site containing a stand was substituted into the survey. GPS coordinates were recorded for each surveyed stand.

Stand-level health and infestation densities

Stand mortality was assessed in terms of stand-wide foliage loss, which I rated on a 1-4 scale (1 = 0-25% loss; 2 = 26-50% loss; 3 = 51-75% loss; 4 = 76-100% loss). At each stand, I collected data on infestation densities for *A. tsugae*, *F. externa*, and *O. ununguis*. I haphazardly selected 50 hemlocks (≥ 2 m in height and ≥ 8 cm DBH) located within each stand to assess infestation densities.

Herbivore densities were assessed on each tree by selecting two 1m-long branches, located on opposite sides of the tree whenever possible. On each branch, following the methods of Gómez et al. (2014), I rated the densities of *A. tsugae* and *F. externa* on each tree using a 0-3 scale (0 = none; 1 = 1-10 individuals/m branch; 2 = 11-100 individuals/m branch; 3 = >100 individuals/m

branch). Similarly, I took a measurement for *O. ununguis*, which I rated on a 0-3 scale (0 = none; 1 = 1-10% discolored foliage/m branch; 2 = 11-25% discolored foliage/m branch; 3 = 26-100% discolored foliage/m branch). These measures were then averaged to obtain a tree-level estimate of infestation for each insect. Density data from each of the 50 surveyed trees at each stand were then pooled to produce mean density ratings for *A. tsugae*, *F. externa*, and *O. ununguis*.

Statistical analysis

I fit linear models and used backward-selection to examine how herbivore interactions and latitude shape herbivore densities across the longitudinal transect surveyed. In these models, predictor variables included *A. tsugae* density, *F. externa* density, and *O. ununguis* density and latitude, with competitor herbivore densities as response variables. Using a similar approach, I also examined the effects of these herbivores on hemlock defoliation, my proxy for stand health. Models were ranked using Akaike Information Criterion (AIC) scores. Finally, I performed Pearson's product-moment correlations to test for simple associations between *A. tsugae*, *F. externa*, and *O. ununguis* densities. All analyses were performed using R (Version 3.2.0; The R Foundation for Statistical Computing, 2015), running in RStudio (Version 0.99.441; RStudio Inc., 2015).

Results

Hemlock stand health

Average hemlock defoliation was low to moderate throughout the study area (Table 1.1). In MA and CT, defoliation was low to moderate, (mean density \pm SE: 1.47 ± 0.02 on a scale of 1 to 4), while defoliation was low at every stand throughout the sampled region of NH/VT (1.00 ± 0.00). Excluding NH and VT, the vast majority of stands (90%, $n = 27$) experienced either low (63.3%) or moderate (26.7%) defoliation. No stands in the study area experienced very high defoliation. Backward model-selection used to examine hemlock defoliation revealed a best-fit model with two main effects: *A. tsugae* and *O. ununguis* density (Table 1.2). Stands with higher average *A. tsugae* density had significantly higher defoliation ($P < 0.01$). Average *O. ununguis* density, while a predictor of hemlock defoliation, was not significant ($P = 0.1$).

Insect and mite densities

Adelges tsugae densities were low throughout the study area, as almost half of all sampled stands had low density ratings for this herbivore (48.9%, $n = 22$; Table 1.1). In the NH/VT region, *A. tsugae* was nearly nonexistent, as 80% of the sampled stands ($n = 12$) did not contain any of the insects. The remaining 20% of stands in NH/VT had low *A. tsugae* densities. For the rest of the sampled region, *A. tsugae* densities were low overall (0.78 ± 0.02). The majority of these stands had low densities (63.3%, $n = 19$), and on average, nearly half of the trees in each stand were infested with some density of *A. tsugae* ($46.6\% \pm 3.3$). Backward model-selection used to examine *A. tsugae* density across the surveyed transect revealed a best-fit model with one main effect, *F. externa* density, as well as one interactive effect, *F. externa* density x latitude (Table 1.2). Scale density

was a significant predictor of *A. tsugae* density ($F_{1,41} = 3.99$, $P = 0.05$), though this effect varied with latitude ($F_{1,41} = 3.95$, $P = 0.05$). A Pearson's product-moment correlation also revealed that the presence of higher *F. externa* densities was significantly associated with higher *A. tsugae* densities ($r = 0.63$, $n = 43$, $P < 0.05$). Finally, the presence of *F. externa* at lower latitudes was a predictor of *A. tsugae* (Fig. 1.1).

Fiorinia externa densities were moderate for the entire study area (1.53 ± 0.03 ; Table 1.1). In NH/VT, densities were negligible, as nearly three-quarters of the stands did not contain any *F. externa* (73.3%, $n = 11$), and the remaining stands had low densities (26.7%). The insect was found at high densities throughout MA and CT (2.28 ± 0.02), with 76.7% ($n = 23$) of stands in these two states containing *F. externa* at high densities. On average, the vast majority ($92.9\% \pm 2.9$) of trees in the MA and CT stands were infested. Backward model-selection used to examine *F. externa* densities across the surveyed transect revealed a best-fit model with one main effect, *A. tsugae* density, as well as an interactive effect, *A. tsugae* density x latitude (Table 1.2). *Fiorinia externa* was found at higher densities in stands with higher densities of *A. tsugae*. Moreover, their densities also varied along the latitudinal transect (Fig. 1.1), decreasing in density from south to north. However, in the best-fit model, neither *A. tsugae* ($F_{1,41} = 0.08$, $P = 0.78$; Table 1.2) nor the interactive effect ($F_{1,41} = 0.00$, $P = 0.95$) were significant predictors.

Oligonychus ununguis densities were low throughout the study area (0.48 ± 0.01 ; Table 1.1) and this was fairly homogenous across the entire surveyed area.

MA and CT stands contained more trees infested with *O. ununguis* on average ($46.1\% \pm 2.1$) than stands in NH ($26.7\% \pm 4.0$). However, *O. ununguis* was found at moderate densities more often in NH/VT (20%) than in the rest of the study area (6.7%). Every sampled stand in MA and CT was infested by *O. ununguis* at some density level; this was not the case in NH/VT. Backward model-selection used to examine *O. ununguis* densities revealed that the best-fit model had a main effect (*A. tsugae* density) and interactive effect (*A. tsugae* density x latitude), akin to the *F. externa* model (Table 1.2). Like *F. externa*, *O. ununguis* was found at higher densities at lower latitudes and at stands with higher *A. tsugae* densities (Table 1.1, Fig. 1.1). However, neither the *A. tsugae* ($F_{1,41} = 0.05$, $P = 0.83$) nor the interaction with latitude ($F_{1,41} = 0.00$, $P = 0.95$) were significant predictors of *O. ununguis* density (Table 1.2). A Pearson's product-moment correlation revealed though that the presence of higher *O. ununguis* densities was significantly associated with higher *A. tsugae* densities ($r = 0.34$, $n = 43$, $P < 0.05$). *O. ununguis* was also positively associated with *F. externa* ($r = 0.25$, $n = 43$, $P = 0.1$), although this was not significant.

Discussion

This landscape survey revealed that the populations of the two previously surveyed invasives continue to fluctuate in southern New England, while remaining largely nonexistent in NH and VT. In 2011, stands surveyed by Gómez et al. (2014) containing *A. tsugae* had on average ~36% of all trees infested by the

insect; in 2015 that number rose to ~46%. Likewise, stands surveyed in 2011 containing *F. externa* had on average ~77% infested trees, and that number rose to ~93% for the subset of trees sampled in the same region in 2015. From 1997-2011, average *A. tsugae* densities across the surveyed region decreased from 1.45 to 0.62; in 2015, the average *A. tsugae* density was 0.78. During the same period from 1997-2011, average *F. externa* densities increased from 0.37 to 1.81; in 2015 the average density was 2.28 for the subset of trees sampled. While *A. tsugae* populations experienced a small increase from 2011 to 2015, the herbivore is likely continuing its downward trend. Historically, populations have undergone yearly fluctuations due to negative density-dependent feedback; *A. tsugae* populations rise and fall dramatically from year to year as a result of intraspecific competition (McClure 1991). On the whole though, populations have begun to decrease as hemlock stands continue to die off (Gómez et al. 2014). Another factor likely contributing to lower *A. tsugae* densities is the continued increase in populations of *F. externa*; as an effective competitor to the adelgid, this herbivore saw a dramatic rise in average stand densities between 1997 and 2011, and this trend continued in 2015.

While the two invasive herbivores inhabited stands throughout MA and CT, they were largely absent in stands further north than the border of MA and NH/VT. This is most likely due to cold winter temperatures, which continue to be a limiting factor to range expansion of these two insects (McClure 1989a; Paradis et al. 2007). Several stands north of the border did contain *A. tsugae* and *F. externa*, albeit at low densities, which could indicate that either (a) some insects

are evolving cold tolerance, as observed in (Preisser et al. 2008); (b) they are increasingly able to live in stands further north, probably because of a warming global climate; or (c) some combination of those and other factors. However, while climate change and evolved cold tolerance may contribute to the northward expansion of *A. tsugae* and *F. externa* both now and in the future, it appears that neither have progressed to a point where significant expansion is possible at the current time.

Overall, *A. tsugae* was found to be a predictor of *F. externa*, and vice versa; however this is more likely correlative than causative for 2015. Past research indicates that feeding by *A. tsugae* does not necessarily facilitate higher densities of *F. externa*; likewise, *F. externa* has been shown to outcompete *A. tsugae*, resulting in lower densities for the latter herbivore. The present pattern of positive association between the two is more likely due to past colonization history than direct facilitation. It remains possible that there is a facilitative effect of *F. externa* on *A. tsugae* (or vice versa), but the data from this survey alone is insufficient to point to that as a likely explanation. Similarly, while latitude was shown to affect *A. tsugae* and *F. externa* densities, it is unclear whether this effect was truly due to some factor of latitude, or could rather be attributed to the historical pattern of range expansion by the two herbivores. As the North American infestations of both *A. tsugae* and *F. externa* originated south of New England and spread northward, stands at lower latitudes have in general been exposed to each insect for longer periods of time. Further research involving

simultaneous inoculations of the pests into stands at different latitudes could help to parse out these two factors.

This landscape survey was the first of its kind to quantify the densities of *O. ununguis* in hemlock stands throughout New England. Overall, densities were low (0.48), with ~40% of the trees in each stand infested on average. Every stand in both MA and CT was infested with the mites, but the average densities of *O. ununguis* in those two states were lower than those found in NH and VT. One possible explanation for this is that the higher human populations density in MA and CT compared to NH and VT may contribute to the spread of *O. ununguis*. As a non-specific feeder and common horticultural pest, *O. ununguis* is likely present in many gardens and fragmented forests in southern New England, contributing to its widespread infestation on hemlock. However, the low overall *O. ununguis* densities, coupled with the higher average densities of *A. tsugae* and *F. externa* throughout this region, suggest that mites may not be able to compete well with the invasive herbivores on those trees where they co-occur. On the other hand, the best-fit model suggested that *O. ununguis* densities are explained to some extent by the densities of *A. tsugae*. Looking solely at the association between the two, an alternative explanation is possible, as Alba et al (2015) have suggested that spider mites are able to suppress plant defenses, resulting in possible benefits for competing herbivores.

Applying this observation to a two-year scenario, initially high *O. ununguis* populations in an area during year one would result in widespread suppression of plant defenses that year and into the next. During year two, one

could expect to find increased populations of competitors such as *A. tsugae* and *F. externa*. This fits in with observations of fluctuating populations for those herbivores from 1997-2015. In this context, the NH/VT stands surveyed in 2015 may currently be in ‘year one.’ The higher population densities of *O. ununguis* in those stands could then be attributed to the fact that the ranges of the competing herbivores, *A. tsugae* and *F. externa*, have not yet significantly expanded that far north. Continued observation of the population dynamics for each of the three herbivores may provide evidence to support this hypothesis. This is especially true given that warming climate and potential evolved cold tolerance for the two herbivores will increasingly facilitate the expansion of their ranges in future years, likely leading to higher measurable densities throughout New England.

In this survey, mottling of needles was measured as a proxy for presence of *O. ununguis*, given that mite populations collapse in the summer (Lehman 1998; Lizotte et al. 2012) and the survey was constrained to the summer months. As a result, the measurements of *O. ununguis* densities taken in this survey may form a slightly lagged picture of true current densities and population distribution, since the chlorosis of needles which results in the mottled look is permanent for the duration of the time the needles remain on the tree. Thus, the mottling may have been caused by mites a year or two prior to the survey measurements. This relatively low possible discrepancy between damage and measurement dates means that the mite densities measured in this survey can be seen as a reasonable estimate of population densities, but the possible limitations of this approach should be noted.

While the focus of the present survey was on interspecific interactions, the measurements of stand-wide defoliation supported previous work showing that *A. tsugae* is the leading cause of hemlock decline in New England, as *A. tsugae* densities were positively associated with defoliation. *O. ununguis* was associated with higher defoliation; while not significant, these results indicate that mite infestations may be contributing to the decline of hemlock in the surveyed region. This is consistent with previous studies which have shown the damaging effects of mites on conifers. Alternatively, *O. ununguis* might be found at higher densities in MA and CT because of the greater opportunity for dispersal associated with more proximate human development compared to NH and VT. This problem can likely be best explained by further experimental research regarding the effects of *O. ununguis* on the physiology and health of infested *T. canadensis*.

The population dynamics of herbivores are fluid and complex, and when multiple herbivores share a common host, population densities often change in response to inter- and intraspecific competition and facilitation. My results underscore the importance of taking a whole-systems approach to studying the effects of an invasive herbivore, like *A. tsugae*. Future research would likely benefit from such an approach, allowing for a more accurate understanding of the variety of factors influencing the range expansion of harmful invasive species. Land managers too, armed with the knowledge to predict likely effects of an infestation on other herbivore populations, would gain a greater ability to mitigate overall damage.

Table 1.1. Biotic characteristics of New England *Tsuga canadensis* stands surveyed in 2015.

Variable	Score in 2015 (all stands)	Score in 2015 (MA/CT only)	Score in 2015 (NH/VT only)
Sampled stands	45	30	15
<i>Tsuga canadensis</i>			
Hemlock defoliation (1-4)	1.31 ± 0.01 (SE)	1.47 ± 0.02	1.00 ± 0.00
Stands with low (1) defoliation	75.6 % (n = 34)	63.3 % (n = 19)	100 % (n = 15)
Stands with moderate (2) defoliation	17.8 % (n = 8)	26.7 % (n = 8)	0 % (n = 0)
Stands with high (3) defoliation	6.7 % (n = 3)	10.0 % (n = 3)	0 % (n = 0)
Stands with very high (4) defoliation	0 % (n = 0)	0 % (n = 0)	0 % (n = 0)
<i>Adelges tsugae</i>			
<i>A. tsugae</i> density (0-3)	0.53 ± 0.02	0.78 ± 0.02	0.01 ± 0.00
Stands with no (0) <i>A. tsugae</i>	31.1 % (n = 14)	6.7 % (n = 2)	80.0 % (n = 12)
Stands with low (0.01-1) <i>A. tsugae</i> density	48.9 % (n = 22)	63.3 % (n = 19)	20.0 % (n = 3)
Stands with moderate (1.01-2) <i>A. tsugae</i> density	13.3 % (n = 6)	20.0 % (n = 6)	0 % (n = 0)
Stands with high (2.01-3) <i>A. tsugae</i> density	6.7 % (n = 3)	10.0 % (n = 3)	0 % (n = 0)
Percentage of <i>A. tsugae</i> infested trees in a stand	31.3 % ± 2.0	46.0 % ± 3.3	1.0 % ± 0.7
<i>Fiorina externa</i>			
<i>F. externa</i> density (0-3)	1.53 ± 0.03	2.28 ± 0.02	0.04 ± 0.01
Stands with no (0) <i>F. externa</i>	24.4 % (n = 11)	0.0 % (n = 0)	73.3 % (n = 11)
Stands with low (0.01-1) <i>F. externa</i> density	13.3 % (n = 6)	6.7 % (n = 2)	26.7 % (n = 4)
Stands with moderate (1.01-2) <i>F. externa</i> density	11.1 % (n = 5)	16.7 % (n = 5)	0 % (n = 0)
Stands with high (2.01-3) <i>F. externa</i> density	51.5 % (n = 23)	76.7 % (n = 23)	0 % (n = 0)
Percentage of <i>F. externa</i> infested trees in a stand	63.5 % ± 2.8	92.9 % ± 2.9	3.2 % ± 1.4
<i>Oligonychus ununguis</i>			
<i>O. ununguis</i> density (0-3)	0.48 ± 0.01	0.54 ± 0.01	0.35 ± 0.03
Stands with no (0) <i>O. ununguis</i>	8.9 % (n = 4)	0 % (n = 0)	26.7 % (n = 4)
Stands with low (0.01-1) <i>O. ununguis</i> density	82.2 % (n = 37)	93.3 % (n = 28)	53.3 % (n = 8)
Stands with moderate (1.01-2) <i>O. ununguis</i> density	8.9 % (n = 4)	6.7 % (n = 2)	20.0 % (n = 3)
Stands with high (2.01-3) <i>O. ununguis</i> density	0 % (n = 0)	0 % (n = 0)	0 % (n = 0)
Percentage of <i>O. ununguis</i> infested trees in a stand	39.7 % ± 1.4	46.1 % ± 2.1	26.7 % ± 4.0

Table 1.2 Main and interactive effects of herbivore densities (*Adelges tsugae*, *Fiorina externa* and *Oligonychus ununguis*) and latitude on competitor densities and host plant health in New England *Tsuga canadensis* stands surveyed in 2015. Backwards model selection was used and models were ranked based on Akaike Information Criterion (AIC) values. The two best models are displayed for each category.

Response variable	<i>A. tsugae</i> density			<i>F. externa</i> density			<i>O. ununguis</i> density		
<i>Effect</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>
<i>F. externa</i> density	1, 41	3.99	0.05						
<i>F. externa</i> density x latitude	1, 41	3.95	0.05						
<i>A. tsugae</i> density				1, 41	0.08	0.78	1, 41	0.05	0.83
<i>A. tsugae</i> density x latitude				1, 41	0.00	0.95	1, 41	0.00	0.95

Response variable	<i>T. canadensis</i> defoliation		
<i>Effect</i>	<i>df</i>	<i>F</i>	<i>p</i>
<i>A. tsugae</i> density	1, 41	19.77	0.01
<i>O. ununguis</i> density	1, 41	2.78	0.10

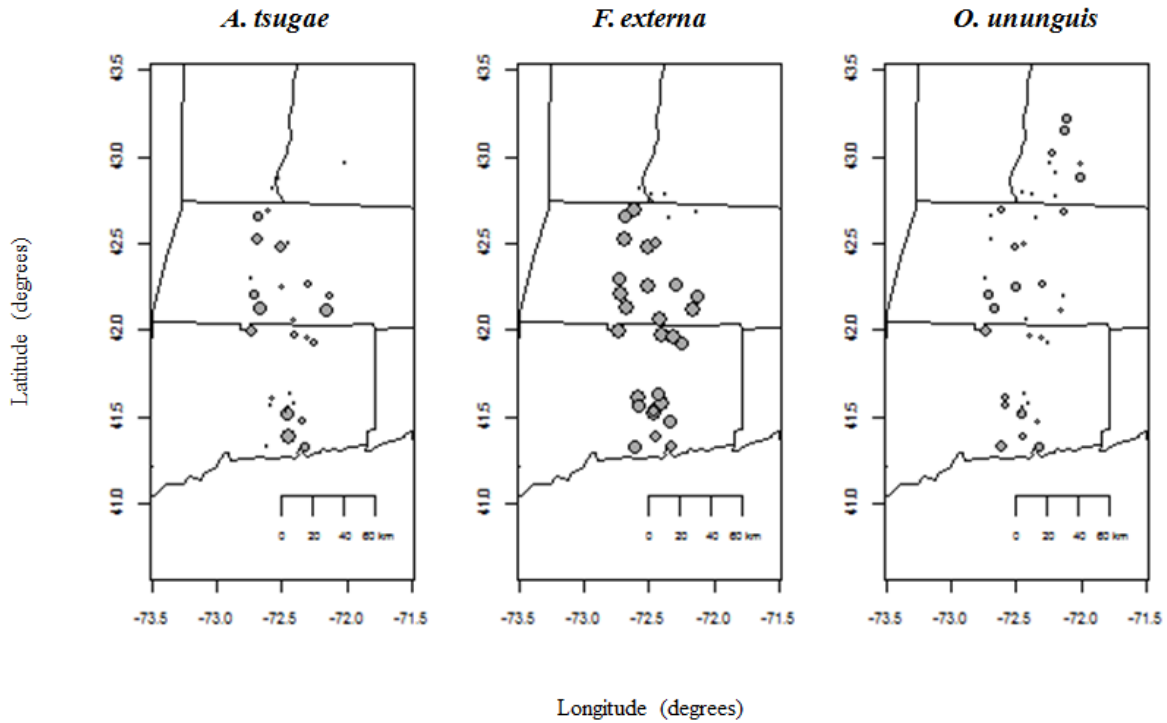


Fig. 1.1 Mean densities of *Adelges tsugae*, *Fiorina externa*, and *Oligonychus ununguis* in New England *Tsuga canadensis* stands surveyed in 2015. Each circle represents a sampled stand along a longitudinal transect across NH, MA and CT. Circle size (increasing) corresponds to average stand-level insect or mite density ratings. Density ratings were scored from 0 to 3, where 0 is no insect or mite damage present. For *A. tsugae* and *F. externa*, 1 is 1–10 insects/m branch, 2 is 11–100 insects/m branch and 3 is 100 insects/m branch. Mottled foliage resulting from herbivory by *O. ununguis* was used as a proxy for *O. ununguis* density; 1 is 1-10% mottled foliage/m branch, 2 is 11-25% mottled foliage/m branch and 3 is 26-100% mottled foliage/m branch. Average densities are based on 50 sampled trees per stand.

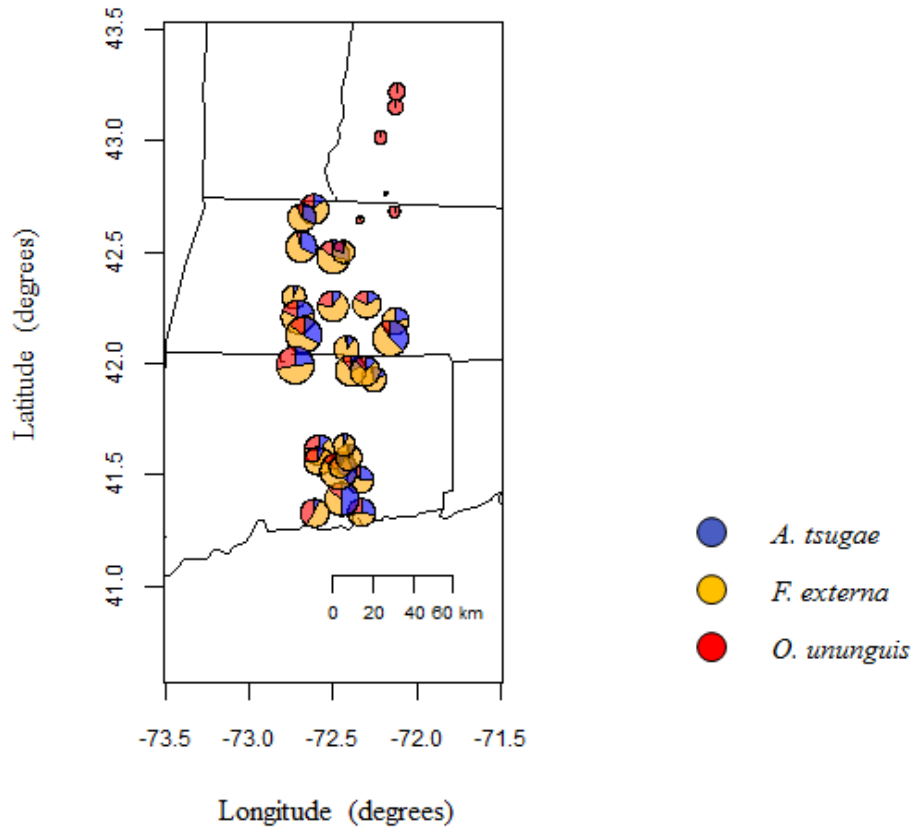


Fig. 1.2 Relative densities of *Adelges tsugae* (blue), *Fiorina externa* (yellow), and *Oligonychus ununguis* (red) in New England *Tsuga canadensis* stands surveyed in 2015. Each pie represents a sampled stand along a longitudinal transect across NH, MA and CT. Pie size (increasing) corresponds to average total stand-level density ratings for the three herbivores combined.

Chapter 2: Competitor abundance and insecticide applications affect herbivore densities on eastern hemlock

Introduction

In a system with multiple herbivores on a shared host plant, competition often arises over access to the most desirable food resources. Plant tissue quality can differ between hosts, but in the case of passively dispersed, sessile herbivores such as *Adelges tsugae*, *Fiorina externa*, and *Oligonychus ununguis*, the fight for food (and by extension, reproductive success) often occurs at the within-host level. In such a situation, one herbivore can alter the quality of resources available to its competitors, either enhancing nutrients in plant tissue or inducing defenses and making tissue less palatable (Gómez et al. 2012). If this were the only factor affecting the nutritional value of available tissue, it would follow that individuals would prefer co-occurrence with those herbivores that enhance nutrient quality, and avoid co-occurrence when defenses are induced. Indeed, it has been shown that *A. tsugae* avoids co-occurrence with *F. externa* (Gómez et al. 2014), as *F. externa* is an effective competitor in these situations, often resulting in reduced densities of *A. tsugae* (Preisser et al. 2007; Miller-Pierce and Preisser 2012; Gómez et al. 2014). Changes to host plant tissue quality brought about by competitors certainly influence the success of individual herbivores, but other factors such as human management can play a role as well.

The decline of eastern hemlock in North America at the hands of the invasive herbivores *A. tsugae* and *F. externa* has resulted in habitat loss and

changes to forest ecosystems and their function (Orwig and Foster 1998; Jenkins et al. 1999; Stadler et al. 2005; Orwig et al. 2008; Cobb 2010). Hemlock loss stands affects humans as well. For example, losses in property values due to the decimation of eastern hemlock by *A. tsugae* have been estimated at a total over \$24 million in southern New England alone (Li et al. 2014). Furthermore, hemlocks are valued for their aesthetic value, and are attractive both in personal gardens and national and state parks (Abella 2014). From a more practical standpoint, dying trees present a safety hazard as falling branches and debris can become unsafe (Abella 2014). Because of their importance to humans and natural ecosystems, decision-makers and land managers often attempt to control for these pests. Since *A. tsugae* - the higher priority threat - does not have any natural enemies in its invaded range, a common method of control is the application of insecticides to affected trees. Imidacloprid, a neonicotinoid, is a widely used insecticide and its use on hemlocks to control for *A. tsugae* has been documented; depending on the type of application, imidacloprid has been shown to effectively suppress *A. tsugae* populations for 1-2 years or more (Webb et al. 2003; Cowles et al. 2006; Frank and Lebude 2011), allowing for significant recovery and regrowth by heavily damaged trees (Webb et al. 2003).

One limitation of imidacloprid application is that while it may be effective against *A. tsugae* (Webb et al. 2003; Cowles et al. 2006; Abella 2014; Benton et al. 2015), it may not be nearly as effective against other undesirable herbivores in the system. Worse yet, unpredicted non-target effects may often arise, such as the increased success of other herbivores on the same host plant (Frank and Lebude

2011). Such effects can be unwanted, such as the harmful impacts to beneficial insects within the ecosystem (Kreutzweiser et al. 2009; Pisa et al. 2014), to whom imidacloprid is toxic. Not all herbivores are equally susceptible to any given insecticide, and so applying imidacloprid can lead to the enhanced success of herbivores – which have a greater tolerance for the chemical – when their competitors are killed off. Positive or desirable non-target effects are could feasibly occur as well, such as the removal of secondary pests in addition to targeted species, but specific effects are likely to differ on a regional basis.

It has been shown that use of imidacloprid can lead to increased fecundity in spider mites, including spruce spider mite (*Oligonychus ununguis*; SSM) in systems including hemlock (James and Price 2002; Raupp et al. 2004; 2008; Szczepaniec 2009; Smith et al. 2013). Several mechanisms have been proposed to explain this. It is possible that imidacloprid directly stimulates the reproductive functions of mites, a phenomenon known as hormoligosis, although evidence of this on mites has been mixed (Raupp et al. 2004). Alternatively, the mites' success might be due to the removal of predators or competitors to whom imidacloprid is toxic (Sclar et al. 1998; Raupp et al. 2004). A third possibility is that imidacloprid alters the tissue quality of the host plant in such a way that leads to more available nutrients for mites. This has been previously suggested (Raupp et al. 2004), and it has been shown that imidacloprid applications disrupt plant defenses (Szczepaniec et al. 2013) in corn, cotton and tomato plants. A similar phenomenon could be occurring on eastern hemlock. From a management perspective, the facilitative effect of imidacloprid on mite performance is

unwanted regardless of the specific mechanism, but for the mites it represents another factor affecting reproductive success, in addition to considerations such as the co-occurrence of competing herbivores.

Here, I examine the effects of natural resource management practices on population densities of a target pest of eastern hemlock, *A. tsugae*, as well as two non-target secondary pests, *F. externa* and *O. ununguis*. Specifically, I look at whether imidacloprid application contributes to the success of one or both of these secondary pests. To address this, I conducted a field survey of stand-wide herbivore densities at two sites in the Greater Boston Area. At one of the sites, imidacloprid was applied to limit *A. tsugae* populations, while the other site was not managed in this way. Additionally, I examined the settlement preferences of these herbivores at a within-tree level and how preference may be affected by competing herbivores. To achieve this, I conducted a field survey of herbivore densities on individual branches and recorded the frequency of co-occurrence between *A. tsugae*, *F. externa*, and *O. ununguis* at each of the two sites.

Methods

Insect and mite densities in urban forests

In spring 2016, I conducted a survey of two eastern hemlock (*Tsuga canadensis*) stands in the Greater Boston Area of Massachusetts. The two stands were both located within an urban matrix. Criteria for site selection were designed to select sites with as many similarities as possible, such as climate, proximity to

anthropogenic influences (via roads around the sites and trails within them), and presence of the three herbivores. The selected sites were located in the Middlesex Fells Reservation (Fells; Winchester, MA) and the Arnold Arboretum of Harvard University (Arboretum; Boston, MA). The primary difference between these two sites was the use of imidacloprid to control for *A. tsugae* in the Arboretum, and the lack of insecticide use in the Fells. At each of the two stands, I collected data on infestation densities for *A. tsugae*, *F. externa*, and *O. ununguis*. I haphazardly selected 50 hemlocks (≥ 2 m in height and ≥ 8 cm DBH) located within each stand to assess densities. Herbivore densities were assessed on each tree by selecting two 1m branches, located whenever possible on opposite sides of the tree. On each branch, following the methods outlined in Chapter 1, this work, as well as in Gómez et al. (2014), I rated the densities of *A. tsugae* and *F. externa* on each tree using a 0-3 scale (0 = none; 1 = 1-10 individuals/m branch; 2 = 11-100 individuals/m branch; 3 = >100 individuals/m branch). Similarly, I took a measurement for *O. ununguis*, which I rated on a 0-3 scale (0 = none; 1 = 1-10% discolored foliage/m branch; 2 = 11-25% discolored foliage/m branch; 3 = 26-100% discolored foliage/m branch). These measures were then averaged to obtain a tree-level estimate of infestation for each insect. Stand mortality was assessed in terms of stand-wide foliage loss, which I rated on a 1-4 scale (1 = 0-25% loss; 2 = 26-50% loss; 3 = 51-75% loss; 4 = 76-100% loss). Density data from each of the 50 surveyed trees at each stand were then pooled to produce mean density ratings for *A. tsugae*, *F. externa*, and *O. ununguis*. I used independent-samples t-tests to compare densities between the two sites; these analyses were performed using R

(Version 3.2.0; The R Foundation for Statistical Computing, 2015), running in RStudio (Version 0.99.441; RStudio Inc., 2015).

Insects and mites at the branch level

In spring 2016, I conducted two surveys of *A. tsugae*, *F. externa*, and *O. ununguis* feeding site preference in the field. I again selected the Middlesex Fells Reservation and the Arnold Arboretum for the two survey sites. At each site, I haphazardly selected 50 hemlocks, and for each I selected two ~10cm shoots to analyze. For each of these shoots, I counted the total number of needles on each shoot; number of needles with no herbivores; *A. tsugae* only; *F. externa* only; *O. ununguis* only; and each combination of the three when they resided simultaneously. The results from the two shoots were averaged to obtain a value for each tree, and I used 2 x 2 contingency tables and Chi-square tests to analyze the expected distribution of needles in each category for each pairwise herbivore combination. These analyses were performed using VassarStats (Richard Lowry, 2016). I used Fisher R-Z transformations to compare the correlation coefficients of each association between the two sites; these analyses were performed in IFA Services (University of Amsterdam, 2016).

Results

Insect and mite densities in urban forests

Herbivore densities differed significantly between the two sites, though the direction of this difference varied depending on the herbivore. For instance, *A. tsugae* densities were ~195% higher at the Middlesex Fells than at the Arnold Arboretum ($t = 7.34, n = 98, P < 0.01$). HWA densities averaged 2.30 ($SD = 0.90$) individuals/m branch, whereas the Arboretum had densities averaging 0.78 ($SD = 1.14$) individuals/m branch. In contrast, *F. externa* densities were ~14% higher at the Arboretum ($M = 2.72, SD = 0.53$) in comparison to the Fells ($M = 2.38, SD = 0.60$) ($t = -2.98, n = 98, P < 0.01$). Finally, like *F. externa*, *O. ununguis* densities were significantly higher (~211%) at the Arboretum in comparison to the Fells ($t = -11.32, n = 98, P < 0.01$). SSM densities averaged 2.18 ($SD = 0.74$) % discolored foliage/ m branch at the Arboretum, while densities at the Fells averaged 0.70 ($SD = 0.54$) % discolored foliage/ m branch.

Insects and mites at the branch level

The 2 x 2 contingency table analysis used to examine herbivore settlement preference in the Fells revealed that *A. tsugae* and *F. externa* co-occurred less often than expected ($\chi^2 = 21.88, P < 0.0001$; Table 2.1). A random distribution of *A. tsugae* and *F. externa* with respect to each other would have resulted in co-occurrence of the two on 1609 (~16.1%) of the needles; when in fact, the two insects were found together on 1501 (~15%) of the needles. Additionally, *A. tsugae* and *O. ununguis* co-occurred more often than expected ($\chi^2 = 6.72, P = 0.01$). If distributed randomly with respect to each other, the two would have co-occurred on 14 needles when in fact they were found together on 20 needles. Likewise, *F. externa* and *O. ununguis* co-occurred more often than expected ($\chi^2 =$

10.94, $P = 0.001$). The two co-occurred on 12 needles, compared to the expected 6 had they been randomly distributed.

The 2 x 2 contingency table analysis used to examine herbivore settlement preference in the Arboretum revealed that *A. tsugae* and *F. externa* co-occurred more often than expected ($\chi^2 = 81.12$, $P < 0.0001$; Table 2.2). Had they been randomly distributed, the two insects would have co-occurred on 210 (~1.3%) of the needles, when in fact they co-occurred on 300 (~1.9%) of the needles, an increase of 46% over the expected value. *A. tsugae* and *O. ununguis* co-occurred more often than expected as well; however, due to the low ($n = 2$) expected frequency of co-occurrence, a chi-square analysis could not be performed. They were found together on 14 needles, while random distribution would have predicted their co-occurrence on only 2. Likewise, *F. externa* and *O. ununguis* co-occurred more often than expected ($\chi^2 = 114.18$, $P < 0.0001$). Had the two been randomly distributed, they would have co-occurred on 209 (~1.3%) of the needles; in reality, they were found on 316 (~2.0%) of the needles, representing an increase of 53.8% over the expected value.

I used Fisher R-Z transformations to compare the results of the 2 x 2 contingency tables between sites for each association. This analysis revealed that the rate of co-occurrence (compared to expected rate) for *A. tsugae* and *F. externa* was higher at the Fells than the Arboretum ($P = 0.05$). Conversely, the rate of co-occurrence compared to the expected rate for *F. externa* and *O. ununguis* was higher at the Arboretum ($P = 0.03$). The rates for the *A. tsugae* – *O. ununguis*

associations at the two sites could not be compared due to the low expected frequency of co-occurrence at the Arboretum.

Discussion

Contrasting natural resource management practices between the two parks resulted in significant differences in infestation levels of the primary and secondary pests studied. I found evidence in both surveys that application of imidacloprid was successful in reducing *A. tsugae* populations. However, application likely resulted in significant outbreaks of competing *F. externa* and *O. ununguis*. While the mechanisms underlying these shifts in infestation levels remain to be studied, it's clear that management practices can have profound consequences for the success of primary and secondary pests, as well as the health and aesthetic value of eastern hemlock forests.

Adelges tsugae densities were lower in the Arboretum than the Fells, almost certainly due to human management efforts focused on this pest. The Arboretum has a history of managing *A. tsugae*, in which soil applications of imidacloprid have been used to control presence and densities (Arnold Arboretum 2016). Conversely, densities of *F. externa* and *O. ununguis* were found to be higher in the Arboretum than in the Fells. This is likely partially explained by the removal of a widespread competitor, *A. tsugae*, in the Arboretum through the aforementioned use of an insecticide. The effects of imidacloprid itself could also partially explain these results, as *O. ununguis* has been shown to benefit from

applications of imidacloprid, possibly through benefits to reproductive functions (Raupp et al. 2004). Additionally, imidacloprid applications have been shown to increase plant nutritional quality (Raupp et al. 2004), although the precise mechanism has not been well-researched. Different studies have revealed the efficacy of imidacloprid in controlling scale insects to be anywhere from negligible to moderate (Raupp et al. 2008), and it's possible that *F. externa* could be experiencing a net positive effect from insecticide applications if the direct cost of feeding on affected foliage is outweighed by the benefits of competitor removal.

In the Fells, where no imidacloprid was applied, *A. tsugae* and *F. externa* co-occurred less often than expected, which supports the hypothesis that *A. tsugae* is able to actively avoid *F. externa* at the branch level, as demonstrated in previous research (Gómez et al. 2014). However, in the Arboretum, the frequency of co-occurrence between these two insects was higher than expected. This could be due to the extremely high *F. externa* densities throughout the sampled stand; it's possible that historically consistent high *F. externa* population density in the Arboretum has resulted in limited availability of good feeding sites for co-occurring herbivores. Thus, *A. tsugae* may be left with no choice but to settle on needles with *F. externa* as there is no better alternative.

A. tsugae and *O. ununguis* co-occurred more often than expected in both the Fells and the Arboretum. This could be a result of increased nutritional value of plant tissue after feeding by *A. tsugae*, (Raupp et al. 2004), leading to *O. ununguis* preferring to lay its eggs on the previously eaten foliage. Feeding by

some adelgids has been shown to lead to localized increases in nutrient concentration as well as suppressed plant defenses (Miller-Pierce et al. 2010). While this has not been demonstrated with *A. tsugae*, it would make sense given that the results of the surveys in Chapter 1 and this chapter suggest greater densities of *O. ununguis* are found when *A. tsugae* is not present. These findings could mean that while *O. ununguis* may prefer the tissue quality of needles previously exposed to herbivory by *A. tsugae*, newly hatched mites are not able to outcompete *A. tsugae* for resources. It is possible the two do not have a long enough history of co-evolution, and *O. ununguis*' preference for needles containing and fed upon by *A. tsugae* represents a failure to balance the tradeoff between higher nutrient quality and competition with other herbivores on the same host plant. Likewise, this may help explain the finding that *A. tsugae* and *F. externa* co-occurred more often than expected in the Arboretum.

The mite *O. ununguis* is present in hemlock stands throughout New England and its demonstrated success after applications of imidacloprid should be noted by land managers. When considering actions to take in order to reduce populations of an invasive such as *A. tsugae*, it is important to consider the non-target effects of any insecticides or chemicals used, and research should be conducted regionally to this end, such as the studies in Great Smoky Mountains National Park (Benton et al. 2015). Depending on the specific communal makeup of the area, alternative and/or supplemental methods of control might include application of horticultural oils, bifenthrin, and acephate, which have all been shown to control for *A. tsugae* in certain instances (Frank and Lebude 2011).

However, to best inform management decisions, land managers need to be aware of all potentially affected herbivores in the system, and must also consider the effects of removing an herbivore such as *A. tsugae*, as the sudden removal of a competitor can benefit other herbivores sharing the same host. This is nearly always a cost-benefit decision due to the complexity of ecosystems, but research such as this will hopefully provide the necessary knowledge to make the best possible decisions.

Table 2.1 Observed counts of *Adelges tsugae*, *Fiorinia externa*, and *Oligonychus ununguis* on individual needles of eastern hemlock naturally occurring at the Middlesex Fells Reservation, Winchester MA. Numbers in brackets represent the expected values in each pairing.

		<i>A. tsugae</i>	
		Absent	Present
<i>F. externa</i>	Absent	3191 (3299)	3764 (3656)
	Present	1559 (1451)	1501 (1609)

		<i>A. tsugae</i>	
		Absent	Present
<i>O. ununguis</i>	Absent	3191 (3185)	3764 (3770)
	Present	5 (11)	20 (14)

		<i>F. externa</i>	
		Absent	Present
<i>O. ununguis</i>	Absent	3191 (3185)	1559 (1565)
	Present	5 (11)	12 (6)

Table 2.2 Observed counts of *Adelges tsugae*, *Fiorinia externa*, and *Oligonychus ununguis* on individual needles of eastern hemlock naturally occurring at the Arnold Arboretum, Boston MA. Numbers in brackets represent the expected values in each pairing.

		<i>A. tsugae</i>	
		Absent	Present
<i>F. externa</i>	Absent	7786 (7696)	113 (203)
	Present	7846 (7936)	300 (210)

		<i>A. tsugae</i>	
		Absent	Present
<i>O. ununguis</i>	Absent	7786 (7774)	113 (125)
	Present	95 (107)	14 (2)

		<i>F. externa</i>	
		Absent	Present
<i>O. ununguis</i>	Absent	7786 (7679)	7846 (7953)
	Present	95 (202)	316 (209)

Conclusion

The results of the present research indicate that while spruce spider mite has largely resided in the shadow of research on the two invasive herbivores, HWA and EHS, it is found throughout New England in natural forests as well as urban parks. On its own, mite damage to eastern hemlock may be of little ecological concern, as the primary impacts of SSM alone are mostly aesthetic ones. However, when multiple herbivores share a host as is the case with HWA, EHS and SSM, their combined effects – both on host plant physiology and on each other – may be difficult to predict. Interspecific relationships, both competitive and facilitative, should be taken into consideration by researchers when studying these herbivores. As the ranges of HWA and EHS likely expand northward in the future, they will continue to coincide with SSM in those areas; populations of the three should be monitored closely.

Surveys in the Arnold Arboretum and Middlesex Fells Reservation support the hypothesis that use of imidacloprid leads to outbreaks of SSM on eastern hemlock. If going forward imidacloprid remains the preferred method of controlling HWA infestations, land managers should strive to remain cognizant of the possible non-target effects of its use. Outbreaks of secondary pests may be unavoidable, but by staying well-informed decision-makers will be able to weigh costs and benefits of chemical control and minimize the unexpected. More research investigating the underlying mechanisms of imidacloprid's effects on various species in this system would be useful, as controlling populations of harmful invasives will remain necessary for the foreseeable future. As human

populations grow and ecosystems are altered time and again, ecologists and land managers will certainly be challenged; for protection of vulnerable species such as eastern hemlock, knowledge of the complex dynamics at play may prove to be critical.

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